to 26 nucleotides in length in a target nucleic acid molecule; and

b) synthesizing a deoxyribonucleic acid molecule comprising first and second substrate binding regions flanking a core region,

wherein said first substrate binding region has a sequence complementary to a first portion of said preselected nucleic acid target sequence,

said second substrate binding region has a sequence complementary to a second portion of said preselected nucleic acid target sequence, and

said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

(II.) RGGCTAGCHACAACGA (SEQ ID NO 122),

wherein said H = T, C or A, and R = A or G.

REMARKS

The above amendments are to correct informalities in the sequence listing, including to add sequence identifiers in the specification where needed. The Applicants appreciates the effort made by the Examiner to point out specific informalities.

In the sequence listing, SEQ ID NO:128 is corrected to reflect the proper sequence provided at page 89, line 9 of the specification. The amendments at page 17, line 8 are to provide the correct designations of orientation for the given sequence in accordance with IUPAC nomenclature. The amendments at page 141, lines 24-26 (Claim 1) and page 145, lines 13-15 (Claim 29) are made to comply with formal sequence requirements. The Applicants believe that the suggested informality in Claim 2 set forth in the Office communication is in error. The Applicants further believe that the suggested informality may refer to formula II of Claim 1. The instant informality is corrected herein. Therefore, the Applicants believe that this response is fully responsive to the Office communication.

No new matter is added. The Applicants respectfully request entry of these amendments and sequence listing.

If there are any fees associated with this request, please charge our Deposit Account 19-0962.

July 17, 2002
Date

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APPENDIX

VERSION WITH MARKINGS SHOWING CHANGES MADE

In The Specification:

At Page 88, line 17, please amend the paragraph as follows:

The re-selections based on the 8-17 and 10-23 molecules involved six different lineages for each motif. Each lineage entailed 5-21 rounds of in vitro selection, differing with respect to the selection protocol and reaction times. All cleavage reactions were carried out in 2 mM MgCl2, 150 mM NaCl, and 50 mM Tris*HCl (pH 7.5) at 37 C. Reaction times varied from 60 min in early rounds to 1 min in later rounds. Each starting pool of templates was based on a sequence complementary to the prototype, with fixed binding arms of seven nucleotides each and a catalytic core randomized to 25% degeneracy at each nucleotide position. For the 8-17 and 10-23 motifs, the templates had the sequence
5'-gtgccaagcttaccgagtaactTCG-TCCGGCTCGGRagatgggtcgtctgtccttccATCT CTAGTTACTTTTTC- 3' and
5'-gttgccaagcttaccg-ggaaaaaTCGTTGTAGCTAGCCtaactaggtcgtctgtccttccA TCTCTAGT TACTTTTTC-3', respectively (PCR primer sites in lower case; substrate-binding arms underlined; randomized positions italicized). The primer used in the template-directed extensions had the sequence
5'-biotin-r(GGAAAAA-GUAACUAGAGAUGG)d(AAGAGATGGCGAC)-3' (SEQ ID NO:132). The PCR primers for the 8-17-based selections were 5'-GTGCCAAGCTTACCGAGTAACT-3' and 5'-d(GGAAGGACAGACGACC-CATC)rU and for the 10-23-based selections were 5'-GTGCCAAGCTTACCGGGAAAAA-3' and 5'-d(GGAAGGACAGACGACCTAGTT)rA. The PCR primers encompassed the binding arms, thus fixing these

sequences. One of the PCR primers in each set contained a 3'-terminal ribonucleotide, allowing isolation of the template strand from the double-stranded PCR products by alkaline hydrolysis of the non-template strand and subsequent purification by polyacrylamide gel electrophoresis. A gel-based selection scheme was employed in some of the lineages. In those cases, the PCR primers were 5'-biotin-GTGCCAAGCTTACCG-3' and 5'-GAAAAAGTAACTAG-AGATGGAAGGACAGACGACC-3' and the extension reactions were carried out on the solid support using the primer 5'-r(GGAAAAAGUAACUAGAGAUGGAAG)-3'. A trace amount of [a-32P]-dATP was included in the mixture to label the extension products, which were eluted with alkali, purified by denaturing polyacrylamide gel purification, and recovered by electroelution. The molecules then were reacted and those that underwent cleavage were isolated by gel electrophoresis.

At Page 59, line 26, please amend the paragraph as follows:

Figure 3 illustrates the sequence alignment of individual variants isolated from the population after five rounds of selection. The fixed substrate domain (5'-GGGACGAATTCTAATACGACTCACTATrAGGAAGAGATGGCGAC-3' (SEQ ID NO:13), or 5'-GGGACGAATTCTAATACGACTCACTATNGGAAGAGATGGCGAC-3', where N represents adenosine ribonucleotide) (SEQ ID NO 13) is shown at the top, with the target riboadenylate identified with an inverted triangle. Substrate nucleotides that are commonly involved in presumed base-pairing interactions are indicated by a vertical bar. Sequences corresponding to the 50 initially-randomized nucleotides are aligned antiparallel to the substrate domain. All of the variants are 3'-terminated by the

fixed sequence 5'-CGGTAAGCTTGGCAC-3' (SEQ ID NO 1) ("primer site"; not shown). Nucleotides within the initially-randomized region that are presumed to form base pairs with the substrate domain are indicated on the right and left sides of the Figure; the putative base-pair-forming (or substrate binding) regions of the enzymatic DNA molecules are individually boxed in each sequence shown. The highly-conserved nucleotides within the putative catalytic domain are illustrated in the two boxed columns.

At Page 61, line 28, please amend the paragraph as follows:

Synthetic DNAs and DNA analogs were purchased from Operon Technologies. The 19-nucleotide substrate, 5'-pTCACTATrAGGAAGAGATGG-3' (SEQ ID NO:7) (or 5'-pTCACTATNGGAAGAGATGG-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 7), was prepared by reverse-transcriptase catalyzed extension of 5'-pTCACTATrA-3' (SEQ ID NO:8) (or 5'-pTCACTATN-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 8), as previously described (Breaker et al, Biochemistry, 33:11980-11986, 1994), using the template 5'-CCATCTCTTCCTATAGTGAGTCCGGCTGCA-3' (SEQ ID NO 9). Primer 3, 5'-GGGACGAATTCTAATACGACTCACTATrA-3' (SEQ ID NO:6) (or 5'-GGGACGAATTCTAATACGACTCACTATN-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 6), was either 5'-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (primer 3a) or 5'-thiophosphorylated with [γ-S]ATP and T4 polynucleotide kinase and subsequently biotinylated with *N*-iodoacetyl-*N'*-biotinylhexylenediamine (primer 3b).

At Page 66, line 2, please amend the paragraph as follows:

In designing the catalytic domain, we relied heavily on the composition of the most reactive variant, truncating by two nucleotides at the 5' end and 11 nucleotides at the 3' end. The 15 nucleotides that lay between the two template regions were left unchanged and a single nucleotide was inserted into the 3' template region to form a continuous stretch of nucleotides capable of forming base pairs with the substrate. The substrate was simplified to the sequence 5'-TCACTATrA • GGAAGAGATGG-3' (SEQ ID NO:12) (or 5'-TCACTATN • GGAAGAGATGG-3', wherein "N" represents adenosine ribonucleotide) (SEQ ID NO 12), where the underlined nucleotides correspond to the two regions involved in base pairing with the catalytic DNA molecule.

At Page 17, line 4, please amend the paragraph as follows:

Figures 4A and 4B illustrate DNA-catalyzed cleavage of an RNA phosphoester in an intermolecular reaction that proceeds with catalytic turnover. Figure 4A is a diagrammatic representation of the complex formed between the 19mer substrate (5[3]'-TCACTATrAGGAAGAGATGG-3[5]', SEQ ID NO 2) and 38mer DNA enzyme (5'-ACACATCTCTGAAGTAGCGCCGCCGTATAGTGACGCTA-3', SEQ ID NO 3). The substrate contains a single adenosine ribonucleotide ("rA", adjacent to the arrow), flanked by deoxyribonucleotides. The synthetic DNA enzyme is a 38-nucleotide portion of the most frequently occurring variant shown in Figure 3. Highly-conserved nucleotides located within the putative catalytic domain are "boxed". As illustrated, one conserved sequence is "AGCG", while another is "CG" (reading in the 5'-3' direction).

In The Claims:

At Page 141, line 4, please amend Claim 1 as follows:

1. A catalytic DNA molecule having site-specific endonuclease activity specific for a nucleotide sequence defining a cleavage site in a preselected substrate nucleic acid sequence,

said molecule having first and second substrate binding regions flanking a core region,

wherein said first substrate binding region has a sequence complementary to a first portion of said preselected substrate nucleic acid sequence,

said second substrate binding region has a sequence complementary to a second portion of said preselected substrate nucleic acid sequence, and

said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

(II.) RGGCTAGCH[X]ACAACGA (SEQ ID NO 122),

wherein said H[X] = T, C or A, and R = A or G.

At Page 144, line 21, please amend Claim 29 as follows:

29. A method of engineering a catalytic DNA molecule that

cleaves a preselected substrate nucleic acid sequence in a target nucleic acid molecule, comprising the steps of:

a) selecting a substrate nucleic acid sequence of from 10 to 26 nucleotides in length in a target nucleic acid molecule; and

b) synthesizing a deoxyribonucleic acid molecule comprising first and second substrate binding regions flanking a core region,

wherein said first substrate binding region has a sequence complementary to a first portion of said preselected nucleic acid target sequence,

said second substrate binding region has a sequence complementary to a second portion of said preselected nucleic acid target sequence, and

said core region having a sequence according to the formula:

(I.) T(stem)'AGC(stem)"Z,

wherein said (stem)' and (stem)" are each three sequential nucleotides which when hybridized as a (stem)':(stem)" pair comprise three base pairs including at least two G:C pairs and wherein said Z = WCGR or WCGAA, and W = A or T and R = A or G; or

(II.) RGGCTAGC<u>H</u>[X]ACAACGA (SEQ ID NO 122),

wherein said <u>H</u>[X] = T, C or A, and R = A or G.